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Pathology of marine warmwater finfish in Israel : problems and research

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Abstract — *A condition in **Sparus aurata** larvae termed « distended gut syndrome » (DGS) has become a major problem in the past three seasons. Unable to digest rotifers and brine shrimp nauplii, the larvae develop a swollen abdomen, exhibit a disoriented spinning motion and are swept passively with the current. Bacterial or viral infections were suspected. Chloramphenicol-supplemented larval food did not affect the prevalence of DGS. Virus-like particles 80 nm in diameter were observed in necrotic cytoplasm of midgut wall cells; however, attempts to grow this putative virus **in vitro** were unsuccessful. Environmental and nutritional factors were also considered, but the etiology of this disease is still unknown. Systemic granuloma, a metabolic disorder of **S. aurata** that causes severe nephropathy and urolithiasis reappeared again in our cultures after a six year interval during which not a single case was diagnosed. The disease was believed to be related to unbalanced, deficient or spoiled diet. In the light of the most recent episodes, however, it is clear that diets alone cannot be held responsible for the conditions. Erythrocytic congestion, lamellar anevrysm and clubbing, trombosis formation, epithelial hyperplasia and degenerative processes were often observed in the gills of nursery grown fish. While these symptoms appeared sometimes in conjunction with identifiable biological agents, (e.g. epitheliocystis clamydia-like organism, **Amyloodinium ocellatum, cryptocaryon irritans**), water supersaturation, stocking density and other environmental factors were often held responsible for their development.*

The rapid development of intensive mariculture worldwide over the past two decades has not been followed by an equally rapid development of veterinary medicine for aquatic animals. This problem is felt even more in tropical mariculture, where tradition does not date back as far as that of cold water species.

The first stocks of gilt-head sea bream *Sparus aurata* were brought to Eilat as fingerlings from Bardawil Lagoon on the Mediterranean coast of Sinai. Some of these fish succumbed to stress during the trip and some soon after their arrival at Eilat due to secondary bacterial infections (Colorni et *al.*, 1981). The great majority, however, survived, pointing at this robust lagoon fish as the right choice for a mariculture industry on the Red Sea. These wild stocks carried a wide array of parasites : over 14 species were identified at the time (Paperna, 1983). The endoparasites

were mainly trematode metacercariae that require a benthic mollusc to complete their development. Since such an intermediate host was absent from our system, these larval helminths gradually disappeared.

Today only ectoparasitic species, such as *Trichodina, Trichodinella* (Ciliata), *Colponema* (Flagellata), and the monogeneans *Gyrodactylus* and *Furnestinia echeneis* (Diplectanidae) proliferate on the fish and, with the exception of *Colponema* whose pathogenicity has not yet been established, still cause some problems. These infections though are sporadic, usually easily diagnosed, and their appearance is dealt with efficiently by formalin treatments. On the contrary, the chlamydia-like organism of epitheliocystis has been responsible for high rates of mortalities in fingerlings in the past years. Epitheliocystis infection occurs in gills of *S. aurata* either as a « benign » infection, with a few or several infected cells (« cysts ») and a limited epithelial tissue response (Fig. 1), or as a « proliferative » hyperinfection with severe hyperplasia of the gill epithelium (Fig. 2) (Paperna, 1977). So far no treatment has been found for this condition.

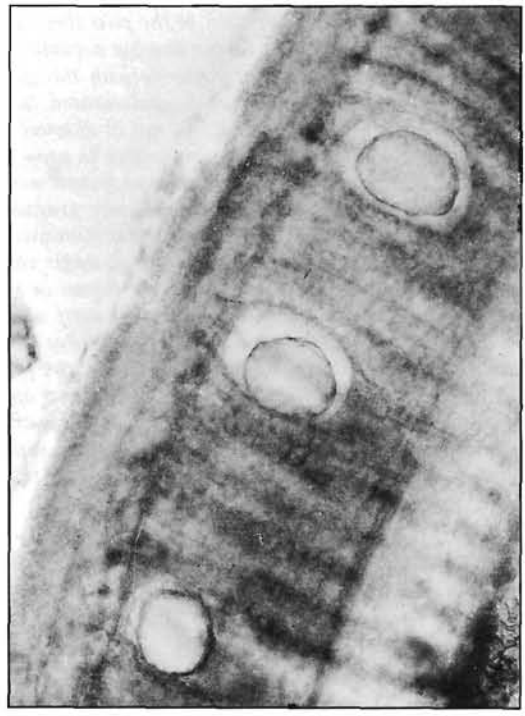

Figure 1. — Gill epitheliocystis, light infection.

Invasive bacteria are occasionally responsible for infections which occur especially after handling operations. The great majority of these bacteria belongs to the genus *Vibrio* and causes acute septicemia in the sea bream and in the other species of fish cultured in Eilat. However, when

healthy fish are challenged with these strains (*V. alginolyticus, V. parahaemolyticus, V. anguillarum* or *anguillarum*-like) by intraperitoneal injection, the fish do not develop clinical symptoms and the bacteria are not recovered from the fish blood. Difficulty in experimentally reproducing septicemic conditions indicates a lack of primary pathogenicity and the requirement of a physiological stress on the host before these strains are able to manifest their opportunism.

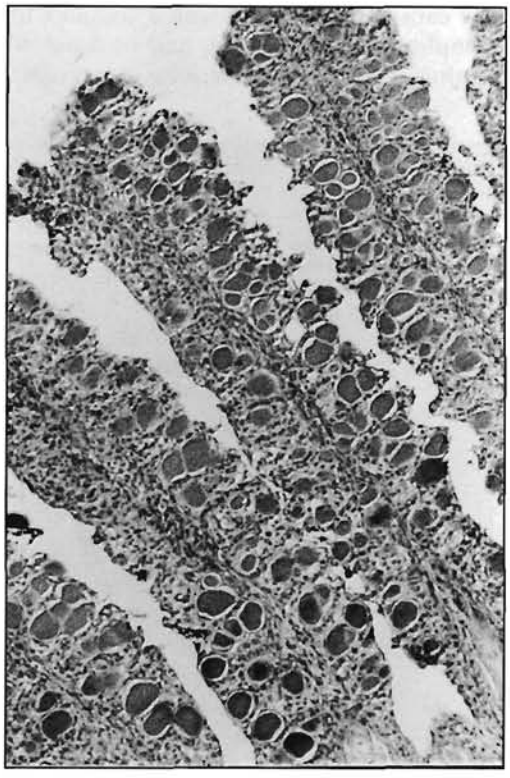

Figure 2. — Gill epitheliocystis, hyperinfection.

Another serious problem affecting practically all open ponds in Eilat is the gas bubble disease. During summer, the intense solar radiation, coupled with the high organic load of the pond water (uneaten food, fish feces, etc.), produces algal blooms and an incessant photosynthesis. Consequently the fishes breathe in a highly oxygenized water all day long. At sunset photosynthesis stops, water exchange continues and the algae start using, rather than producing, oxygen. A rapid external drop in oxygen pressure occurs, with which fishes are not able to cope. Consequently, the fish supersaturated blood fills up with emboli that reach and block capillaries. Downstream tissues are left ischemic, and the consequences become evident in post-mortem examination, in particular of the gills that present conspicuous erythrocytic congestion, telangiectasis, lamellar aneurysm and other degenerative processes. Branchiopathy then predisposes

the fish to infection of microorganisms that are normally kept at asymptomatic levels. Two commercial algicides, Diurex and Terbutrex, were tested but while a limited effect was observed on the algae at the recommended dosages, higher concentrations were severely detrimental to the fish that soon displayed signs of stress (accelerated breathing, darkened color, nervous swimming by the surface) and anorexia for several days after the treatment.

At present, the most troublesome fish diseases in our cultures are of two kinds : a) those caused by parasites with a complex life cycle that can nevertheless be completed in our system, and b) those whose etiology is still unknown and thus we do not know how to prevent.

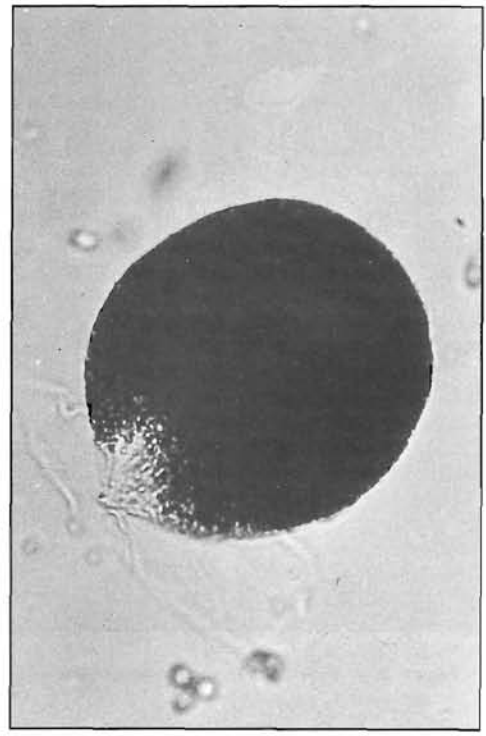

Figure 3. — Trophont of *Amyloodinium ocellatum*.

The dinoflagellate *Amyloodinium ocellatum* (Fig. 3) and the ciliate *Cryptocaryon irritans* (Fig. 4) are two classical examples of the first kind. Although phylogenetically very distant from one another, these two parasites have a similar life cycle, with a parasitic stage on the fish (trophonts), a reproductive stage off the fish (tomonts), and an infective free swimming stage (dinospores and tomites, respectively). No other intermediate host is necessary to close their life cycle, and in the warm water of Eilat, in a confined environment of a tank or of a pond, their proliferation can rapidly reach devastating proportions, undoing within days the work of months or even years. *A. ocellatum* is treated by continuous exposure of the fish to copper sulfate (Paperna, 1984). This

chemical, however, acts relatively slowly, is often toxic at therapeutic concentrations (0.5-1.0 mg/l), and is rapidly sedimented in sea water by magnesium carbonate. *C. irritans* can be treated by rapidly reducing water salinity to at least 10 mg/l for 3 hours four times at 3-day intervals. The efficacy of this treatment lies in the fact that the osmotic shock destroys the tomonts before they can complete their division process (Colorni, 1987). However, this method is practical only when euryhaline fish and limited volumes of water need to be treated. A certain degree of resistance to both parasites has been observed in fish that have survived previous infections, pointing at the possibility of vaccination as the future control method. *A. ocellatum* has been successfully grown *in vitro* on a cell line of catfish gill tissue (Noga, 1987), and large quantities of pure, sterile parasite can be made available as antigens. Parallel experiments are in progress in Eilat with *C. irritans.* As an example of diseases of still unknown etiology, systemic granuloma, a metabolic disorder of *S. aurata* believed to be related to unbalanced, deficient or spoiled diets, reappeared after a six-year interval during which not a single case had been diagnosed.

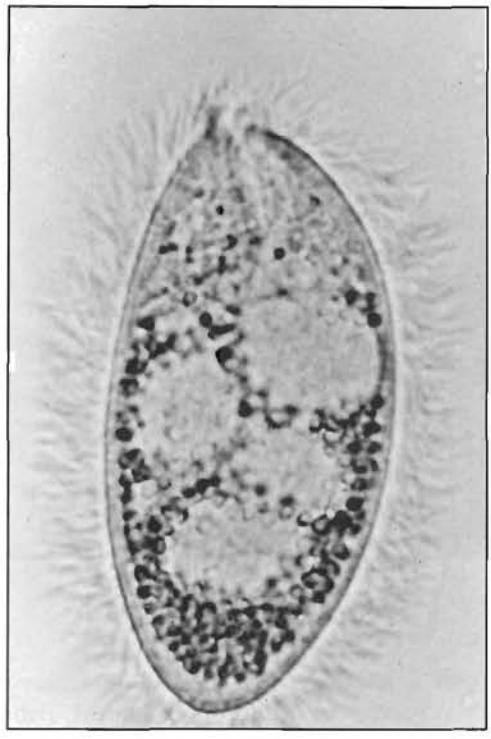

Figure 4. — Tomite of *Cryptocaryon irritans.*

Affected fishes develop severe degenerative changes and necrosis of the renal tubules with eventual collapse of the tubular system (Fig. 5) and formation of granulomata. The condition later spreads to the spleen (Fig. 6) and liver. Severe damage and deformations occur in the eyes (Fig. 7) (Paperna et *al.*, 1980). Presence of viruses, bacteria or fungi has not been detected so far in the affected organs. A similar condition

has been described in the same fish in Spain (Acuigrup, 1983). Quite remarkably, in Eilat this condition affected only *S. aurata* and *Acanthopagrus bifasciatus*, the latter a sparid from the Red Sea that may be considered as the tropical counterpart of the former. During a nutrition experiment carried out in July 1988, a batch of about 300 fishes of 5 to 10 g developed this condition within a short time. The fishes most severely affected, both in number and degree, were the ones that received a high percentage of squid meat in their diet. Degree of severity was arbitrarily determined according to the kidney condition and the characteristic presence in it of tyrosine crystals (Fig. 8) at the first stages of the disease.

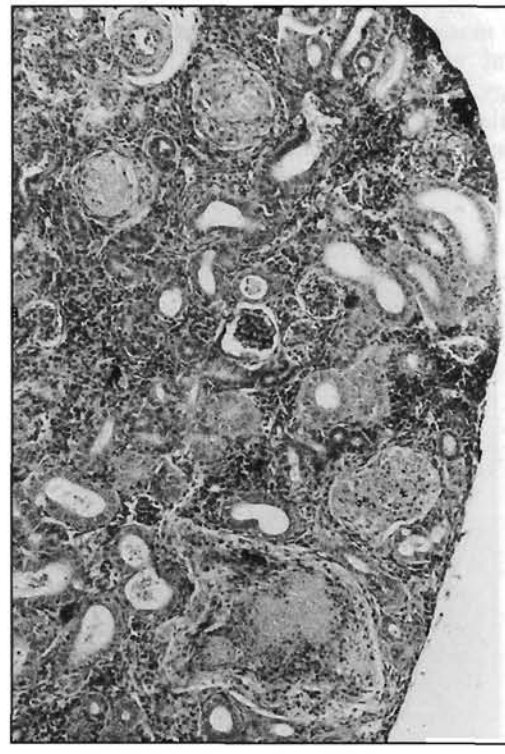

Figure 5. — Granulomatous kidney.

However, altogether a relatively low number of fishes was found to be unaffected in this batch, including the control fishes, whose feeds contained no squid. In the past, empirical change of feeds to fresh diet resulted in regression of the disease incidence which suggested a relation of this condition to dietary problems (Paperna et *al.*, 1980; Acuigrup, 1983). However such relation was never proved and the etiology of this disease is still unknown. More recently, a « granulomatous hypertyrosinemia » was induced in turbot (*Scophthalmus maximus*) fed for over 5 months with an ascorbic acid deficient diet (Messager et *al.*, 1986), while injections of ascorbic acid in the affected fish brought about normalization of tyrosinemia and regression of ocular lesions (Messager, 1986). Vita-

min C is a very labile substance, whose activity is conditioned by time, temperature, oxygen, pH, light, etc. In our feeds it is lipid-coated to prevent its dispersion into the aqueous medium before ingestion by the fish. Although the possibility exists that the vitamin C batch used was somehow faulty, it is puzzling that the same batch has been used for the preparation of other feeds, but no new cases have been diagnosed since this episode.

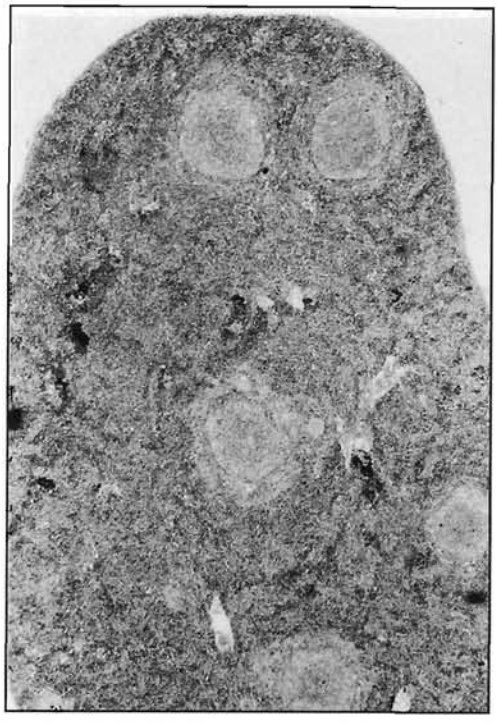

Figure 6. — Granulomatous spleen.

Another condition that affects the gilt-head sea bream was called Distended Gut Syndrome (DGS) and is associated with mass mortalities of the larval stage. To date, the disease has been shown to affect larvae 9 to 32 days old. The afflicted larvae develop a swollen abdomen, exhibit a disoriented, rolling or spinning motion, and are swept passively with the current. In most larvae, the feeding response seems to persist as if out of control, and the alimentary tract becomes engorged with large clumps of undigested or semi-digested rotifers or brine shrimp nauplii. The severely dilated mid and posterior gut contains vast populations of gram negative bacteria, mainly *Vibrio* spp. *Epithelial hyperplasia* is often associated with leukocyte infiltration into the lamina propria of the mid gut. Vacuolation of hepatocytic mitochondria and a marked reduction of zymogen granules in the pancreatic acinar cells are also commonly seen. Microvilli look disrupted and a paucity of pinocytotic vacuoles in the absorptive cells is

evident, indicating an impairment of the normal absorptive processes. Spherical, membrane-bound electron-dense inclusions, approximately 85 nm in diameter were observed in cells of the midgut epithelium. Tissue ultrafiltrates from homogenates of larvae with acute DGS symptoms were inoculated in nine different cell lines, but attempts to grow this putative virus *in vitro* failed. It is noteworthy, however, that the cell lines used in these challenges were all from non sparid fish and it is thus possible that a virus was present but unable to replicate (Diamant, in press).

Figure 7. — Typical appearance of the eye in *S. aurata* affected by systemic granuloma.

Prevalence of body deformities is high in hatchery spawned sea bass while is more moderate in gilt-head sea bream. Its correlation with environmental and nutritional factors, including ascorbic acid deficiency, is presently under study in Eilat. Fishes with opercular deformities, scoliosis, swimbladder defects and other abnormalities demonstrate growth retardation and become very susceptible to bacterial and parasitic infections. These fishes, nicknamed « sentinel fish », are usually the first to show signs of distress or to die when conditions in the system deteriorate. A normal swimbladder is paramount for proper shape and growth. A partial correlation could be established between spinal deformities in gilt-head sea bream fingerlings and non-differentiation of swimbladder epithelium, whose cells, rather than developing into a functional cuboid lining, become hypertrophic and gradually degenerate. Apparently,

swimbladder pressure is important for the normal development of the spine in early life stages. However, scoliosis and non- or partial inflation of the swimbladder can also occur independently in both fishes. The stimuli required to ensure proper development of this organ are presently being studied.

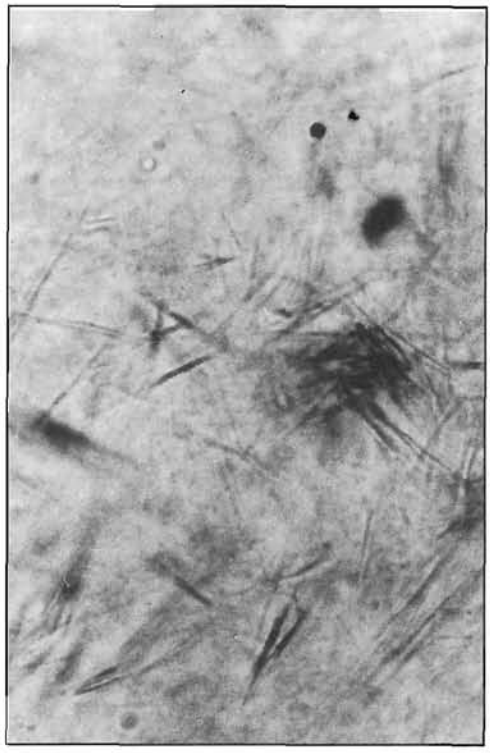

Figure 8. — Characteristic tyrosine crystals from a fresh smear of kidney tissue (early stages of systemic granuloma).

The choice of gilt-head sea bream and European sea bass as the main species to be cultured in Eilat was dictated by both the sturdiness of these two species and the price they can fetch in the European markets. However the natural habitat of these two fishes is the temperate water of the Mediterranean Sea. Higher temperatures have, for example, a strong inhibitory effect on the oogenesis of the sea bass (Y. Zohar, unpubl.), which may be an important limiting factor for the culture of this species in our region. Perhaps some of the deformities or diseases of nuclear etiology simply represent failures of the fish physiology to adapt to the high salinity and temperatures of the Red Sea.

Spontaneous tumors rarely occur in our cultures. A case of lamelo-blastic fibro-odontoma of the lips (Paperna et *al.*, 1977) and a branchial osteochondroma (Nash and Porter, 1985) were diagnosed in *S. aurata*, while a large tumor, probably arising from the thymus gland, was more recently diagnosed in *Dicentrarchus labrax*.

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